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Concerning an Investigation of the Activity of Alkaline Phosphatase in the Blood Serum as an Indicator of the Reactivity of the Organism in the Infectiously Ill.

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Trudy Instituta Mikrobiologii, Akademii Nauk, Latvianskoi SSR.  
5: 57-65: 1957.

Investigators have long noticed the existence of the close relation between the condition of an organism and the function of its ferments. One of the founders of our native enzymology - A. N. Bakh (1923), in relation to this wrote, "hardly anyone can now dispute the contention that between the condition of an organism and the function of its ferments, exists such a close connection that any changes in the condition of the organism is either caused by, or accompanied by a change in the function of the ferments." A. N. Bakh also noticed the close relationship existing between the ferments' activity and immunity. The distinct parallelism, which occurs between the appearances of immunity, on the one hand, and the fermental reactions, on the other, causes many investigators to find here something more general than superficial coincidence (Blagoveshchenskiy, 1941).

At present there is accumulated in science a great quantity of new facts, which permit one to draw a conclusion about the existence of an organic connection between the change in reactivity of the organism to infectious diseases and the activity of its ferments. We shall illustrate this contention with two examples.

Based upon investigations which have been conducted, K. M. Bykov and V. N. Vasyutochkin (1954) express a concept about the fact that the adaptational-trophic function of the nervous system, with which the adaptive reactions of the organism are related, is brought about, to a significant degree, by means of the action on the different enzymatic processes, particularly - on the oxidizing-regenerating processes in the tissues. Bykov and Vasyutochkin regard these processes as the direct biochemical substrate of the nervous trophic system. E. M. Krëps (1945) also considers that changes of the enzymatic reactions in pathological conditions have an adaptive character and are one of the mechanisms of the adaptational-trophic regulation of the functions. At the same time the reorganization of the reactivity of an organism to an infectious disease, from the biological point of view, also represents an unique adaptational reaction, which is regulatable by the nervous system to the changed conditions of the internal environment, to those new conditions in the organism which were added as a result of its interaction with the microbe-pathogen. This allows us to think that changes in the enzymatic reactions occupy an important place among the other processes which occur in the organism during a reorganization of its reactivity.

The second example which illustrates the organic connection between the reactivity of the organism and the function of its ferment is related to the function of regeneration. On the one hand, these substances enter into the structure of many coferments, that is, enter into the composition of the enzymes themselves; and on the other hand, as was shown by the numerous investigations of A. M. Kirkhenshteyn (1955) and his coworkers (Stolygvo, 1948, 1949; Mutseniek, 1954; Indulen, 1954; Berzin' 1955; and others), the vitamins have a large role in the regulation of the phylactic functions of the organism, in the regulation of its immunobiological reactivity.

Thus, there exists a close connection between the reactivity of the organism and the activity of its ferment. In view of this, one can presume that a reorganization of the organism's reactivity produces definite changes in the function of the corresponding fermental systems.

In spite of the obvious value in an investigation of the activity of the ferment as an indicator to the status of an organism's reactivity to infectious diseases, the problem until now is given insufficient attention. There are practically no concrete investigations in the literature that have to do with the study of the interrelations between the activity of the ferment and the reactivity of the organism to infectious diseases.

In connection with this, we decided to verify on concrete material, how perspective is an investigation of the ferment's activity for judgement of the reorganization of the organism's reactivity to infectious diseases.

The alkaline phosphatase of the blood serum was selected as the subject of the investigation. The selection of this ferment is not accidental. Phosphatases are widely spread through the animal and plant worlds and have a great biological importance. These ferment enter into the well-known triad (phosphorus, ferment and function of the cell) which was distinguished by V. A. Engel'gard (1945) as having an important influence on the majority of the physiological processes that are effected in the cell. The phosphatases were successfully investigated also in the pathological processes, and their investigation found a rather wide utilization in clinical practice. Finally, some authors express the hypothesis that the activity level of the phosphatase of the blood reflects, to a known degree, the changes of the organism's reactivity (Landa-Glaz, 1945; Cherfas, 1952; Shumeyko, 1953; Plctnev, 1953).

In order to resolve the question concerning the expediency of an investigation about the utilization of the activity of the phosphatase as a test which reflects the condition of the organism's reactivity to infectious diseases, the following experiments were conducted:

1. An investigation of the influence of some of the physiologically active substances, which alter the condition of the central and the vegetative nervous system, on the activity of the phosphatase of the blood serum.

2. We ascertained the dependence of the phosphatase of the blood on the content in the blood of ascorbic acid - a vitamin which has great importance for the regulation of biological reactivity.

3. A comparison was made of the change in the activity of the phosphatase and the content in the blood of agglutinins, which are generally accepted as a relative indicator of the organism's immunobiological reactivity to infectious diseases.

4. The activity of the alkaline phosphatase of the blood serum was investigated in different clinical forms of typhoid fever. In this we proceeded from the premise that the gravity, length and character of the disease's course is stipulated for the first part by the reactivity of the patient's organism.

The activity of the alkaline phosphatase was investigated by the original method of A. Bodansky (1933). The inorganic phosphorus in the blood was determined by the method of Fiske and Subarrou, as modified by A. E. Braunshteyn (1928).

Where necessary, the results of the investigation were processed by a method of variation-statistic analysis.

In 79% ( $\pm$  3%) of practically healthy people, the activity of the phosphatase ranged within the limits of from 3 to 6 units; the arithmetical mean of the phosphatase activity of the blood in physiological conditions amounts to  $4.95 \pm 0.12$  units.

The interrelations between the activity of the phosphatase in the blood and the condition of the nervous system was investigated in four groups of convalescents after an acute dysentery. Each group consisted of 8 to 11 persons. We tested the substances which act in an inhibiting or exciting manner on the cerebral cortex (bromides, caffeine), and also the mediators of the vegetative nervous system - adrenalin and acetylcholine.

These substances were injected subcutaneously into the organism of the convalescents in solution form, containing 1 mg of adrenalin, or 10 mg of caffeine, or 100 mg of acetylcholine. The activity of the alkaline phosphatase of the blood serum was investigated prior to the injection of the substances and 30 minutes after. The patients took the bromides internally, 2-3 g each, in the course of 5-6 days. The activity of the blood's alkaline phosphatase was also checked prior to and after this.

The results of the investigation are shown in tables 1 and 2.

As seen in table 1, the substances that act excitingly on the cerebral cortex (caffeine) heighten the activity of the alkaline phosphatase of the blood serum. The substances which act in an inhibiting manner on the cerebral cortex (bromides) lower the activity of this ferment. From this same table is seen that an injection of adrenalin - a mediator of the sympathetic nervous system - led to an increase in the activity of the phosphatase of the blood, and an injection of acetylcholine - a mediator of the parasympathetic nervous system - was accompanied by a lowering of the phosphatase activity.

The results which were received confirmed the data of M. B. Bel'chikov (1955) in regards to the influence of bromides and caffeine on the ac-

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ivity of the phosphatase of the blood. At the same time we were able to show that changes in the functional condition of the vegetative nervous system are also accomplished by regular changes in the action of the alkaline phosphatase in the blood serum. The establishment of a connection between the activity of the phosphatase and the condition of the vegetative, and particularly the sympathetic nervous system is the first step on the way to proving the adaptive character of the changes in the activity of the ferments. The data received serves as an additional illustration to the conception developed lately by K. N. Bykov, V. M. Vayyutochkin, E. M. Kreps and others, who regard the changes of the ferments' activity as one of the mechanisms of adaptational-trophic regulation.

As has already been pointed out, the changes of the organism's reactivity to infectious diseases have an adaptive character. The ascertainment of the fact that changes in the activity of the ferments, that of the phosphatase in particular, also have an adaptive character, gives one a reason to assume that a reorganization of the organism's reactivity to infectious diseases is reflected in a change of the activity of the ferments.

It was also noted that the close connection of the ferments with numerous vitamins is another premise which offers an opportunity of investigating the role of the ferments' activity as an indicator of the organism's reactivity to infectious diseases. There was investigated in our work the dependence of the phosphatase's activity on the saturation of the organism with ascorbic acid. E. I. Vinokurov, Ch. A. Danilenko and Z. I. Spilioti (1951) found that the level of phosphatase activity is a sensitive test of the organism's saturation with ascorbic acid.

Furthermore, I. L. Gural' and E. M. Mironova (1952) proposed to determine the activity of the phosphatase in the blood as an indicator of the organism's saturation with vitamin C in dysentery. Both of these indicators were investigated dynamically throughout the course of diseases, in 32 patients with typhoid fever and in 41 patients with dysentery, for a study of the question about the connection in the activity of the blood's alkaline phosphatase with the content of ascorbic acid in the blood, and with the organism's saturation with this vitamin. As a result of the investigations, it was established that there exists in the indicated infectious diseases, a relative parallelism between the activity of the alkaline phosphatase and the ascorbic acid content of the blood. Thus, independently from the original level of the phosphatase's activity (the level of the phosphatase's activity is stipulated by a series of factors, and even with an expressed hypovitaminosis of vitamin C, it can remain rather high), after the injection of ascorbic acid into the organism, a marked increase of the ferment's activity was observed. We did not succeed completely in confirming the data of Gural' and Mironova, according to whom, the determination of the phosphatase's activity can serve as an indicator of the organism's saturation with ascorbic acid in all cases of dysentery. Thus, in the group of patients with acute and chronic dysentery which occurred on a background of accompanying diseases, regardless of the marked hypovitaminosis of vitamin C, the activity of the phosphatase remained at a normal, or even at a raised level. Evidently, in these cases the accompanying diseases themselves (thyrotoxicosis, hypertension, peptic ulcer, gastritis, tuberculosis et al) stipulated the rise in the phosphatase's activity; at the same time the inhibiting effect of the deficiency of ascorbic acid in the organism was masked.

The most distinct parallelism between the phosphatase's activity and the ascorbic acid content in the blood was detected in the patients with the "pure" forms of acute and chronic dysentery. Apparently, only in these cases can a determination of the phosphatase's activity have a practical importance as an indicator of the organism's saturation with vitamin C.

From the viewpoint of interest to us, however, these results show, with sufficient conclusiveness, the dependence of the phosphatase's level of activity on the ascorbic acid content of the blood. The connection which has been established between the phosphatase's activity and vitamin C - the most important regulator of the phylactic reactions of the organism - confirms the expediency of an investigation of the phosphatase's activity for judging the condition of an organism's reactivity to infectious diseases.

Further investigations were devoted to a comparative study of the dynamics of the changes in the activity of the phosphatase in the blood and an agglutination test in patients with typhoid fever; the agglutination test was made with the blood serum of these same patients in the usual order of clinical examination.

The patients were divided into several groups according to the index of the maximum titer of agglutinins for the given case during the disease. The maximum figures for the phosphatase's activity were found in the patients with the seronegative forms of typhoid fever. In each of the subsequent groups, arranged in order of the titer accretion in the Vidal' test, the level of the phosphatase's activity lowered, and the least figures for the ferment's activity were found in the group of patients with the greatest content of agglutinins in the blood.

The above observation indicates the presence of definite correlative connections between the activity of the phosphatase and the content of agglutinins in the blood. Judging by the fact that the titer of agglutinins is one of the generally accepted comparative indicators of the immunobiological reactivity (to say it more correctly: of the intensity of the humoral immunity) of the organism, it is possible to think that the activity of the phosphatase will be appointed for this purpose in clinical practice.

In conclusion we shall present the results of an investigation of the phosphatase's activity in patients with different clinical forms of typhoid fever. As has already been pointed out, we proceed with this out of a conviction that the gravity, duration and character of the course of a disease occur in the first place as dictated by the condition of the reactivity of the patient's organism.

For an elucidation of the question of interest to us, concerning the character of changes in the activity of the phosphatase in its relation to the organism's reactivity, the typhoid-fever patients were conditionally divided into 2 groups.

To the first group were assigned those patients in which the disease took a mild, moderately acute and acute cyclic form. Into the second group went those patients with the acyclic, persistent complications, or recurring forms of the disease.

We arrived at such a division due to the fact that the cyclic course of the disease is usually observed in the patients with a normal reactivity. While the acyclic course usually occurs as stipulated by an impairment (lowering) of the immunobiological reactivity of the patient's organism, that was proven by special experiments of the appropriate immunobiological reactions in the patients with the persistent and relapsing forms of dysentery, typhoid fevers and other diseases (Budzhe, 1954; Ravich-Birger, 1952, and others).

As seen in table 2, in the patients with an usual course (or with usual reactivity) at the height of the disease the activity of the phosphatase would increase correspondingly to the gravity of the case. During the course of the disease, an even lowering of the activity of the phosphatase down to complete normalization was noted prior to the release from the hospital.

Another picture of the dynamics in the changes of the activity of the phosphatase was found in the patients with the acyclic forms of typhoid fevers. Thus, in the patients with the very severe, persistent forms of typhoid fevers, which occur with expressed intoxication, Status typhosus, depression, or other symptoms which indicate - speaking in the clinical language - a reduced reactivity of these patients' organisms, the activity of the phosphatase remained at normal or even reduced figures.

An altered phosphatase "profile" of the blood serum was detected also in the relapsing and complicated forms of the disease. The development of complications and the addition of intercurrent infections in the patients with typhoid fevers were accompanied by a disruption of the usual dynamics of the phosphatase's activity. A significant increase of the phosphatase's activity was noted together with the development of the complication.

In the patients with the relapsing forms of the disease, a significant increase of the phosphatase's activity was also noted in the pre-relapse period. With the onset of the relapse the activity of the phosphatase would rise still more, in order to evenly decrease in the post-relapse period. A decreasing in the titer of the immunological reactions was found by many authors (Budzhe et al) in the pre-relapse period: this to a certain degree permits one to speak of the decrease in the reactivity as the basic cause of the development of relapses in these patients.

Thus, the clinical observations confirm the above cited suppositions as to the presence of definite relationships between the activity of the phosphatase and the immunobiological reactivity of the organism of typhoid fever patients.

The mechanism and the biological significance of the changes in the activity of the phosphatase in dependence on the condition of the organism's reactivity are not described with sufficient clarity. Nevertheless, the conception of the adaptive character in the change of the ferments permits a partial explanation of the significance in the changes which were found.

Apparently, the increase of the activity of ferments in the cases with the lowered immunobiological reactivity has an adaptive character. It is possible to think that in the case of a repression of the specific defense mech-

anism (in the complicated and relapsing forms of the typhoid fevers), the exchange of substances (metabolism) is compensatorily activated, the activity of several fermenta is intensified and particularly that of the alkaline phosphatase of the blood. This activation of the phosphatase evidently has the character of a nonspecific defense reaction.

And only with the very grave, unresponsive forms of the disease, when it is possible to think that not only the specific reactivity is repressed, but also the nonspecific, compensatory defense mechanisms, was there a lack of a rise in the activity of the phosphatase.

Such ideas about the compensatory character in the changes of the course of the enzymatic reactions in the pathologic processes coincide with the opinion held by N. M. Belykov, V. N. Vasyutochkin, Z. A. Kreps and other scientists. It is not without interest to note that a similar concept of the fermenta's role in infectious diseases was expressed more than 40 years ago by A. S. Marutaev (1912). He considered that the fluctuations in the activity of the fermenta in typhoid fever are intended for the struggle with the infection, but not for an active, direct struggle, but for an indirect one - through the support of the normal physiological condition of the organism.

It is understood that all of the cited explanations bear at present a doubly suppositional character, they can be replaced by exact scientific data only in the future, after a further exhaustive study of this problem.

#### Conclusion

1. The injection into the organism of substances which alter the condition of the central and vegetative nervous system, is accompanied by regular changes of the activity of the alkaline phosphatase of the blood system. The injection of adrenalin and caffeine produces an increase in the activity of the phosphatase; bromides and acetylcholine inhibit the activity of this ferment.
2. We ascertained the relative dependence of the activity of the blood's phosphatase on the saturation of the organism with ascorbic acid. In patients with "pure" forms of dysentery, a determination of the phosphatase's activity can serve as an indicator of the organism's saturation with vitamin C.
3. The activity of the phosphatase is found in correlative proportions with the content of agglutinins in the blood of the patients with typhoid fever. The highest figures for the activity of the phosphatase ~~in~~ were found in the patients with a low titer of agglutinins in the blood; the lower figures were in the patients with a high content of agglutinins. ( )
4. With the patients having cyclic forms of typhoid fever, the activity of the phosphatase increases in the acute period proportionally to the gravity of the disease.

In the patients with the acyclic forms of typhus abdominalis (the persistent and relapsing forms), the activity of the phosphatase does not always correspond to the gravity of the disease. In the patients with the relapsing forms

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of typhoid fever, an increase in the activity of the alkaline phosphatase of the blood is found in the pre-relapse period.

5. The experiments which were conducted point out the expediency of a further investigation of the interrelation between the activity of the blood's alkaline phosphatase and the changes in the organism's reactivity to infectious diseases/

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Table 1.

The activity of the alkaline phosphatase of the blood prior to and after injection of some physiologically active substances which alter the condition of the nervous system.

(median data)

The substances under test.	The activity of the phosphatase in units.	
	Prior to injection	After injection
Caffeine	4.42 $\pm$ 0.11	6.19 $\pm$ 0.17
Bromine	5.06 $\pm$ 0.55	3.6 $\pm$ 0.62
Adrenalin	5.16 $\pm$ 0.15	7.81 $\pm$ 0.15
Acetylcholine	5.21 $\pm$ 0.31	3.82 $\pm$ 0.22

Table 2.

The results of the investigation of the activity of the phosphatase in the different groups of patients with typhoid fever.

(median data)

The forms of the disease.	The activity of the phosphatase in the period of the investigation					
	febrile	drop of temperature	pre-relapse	relapsing complications	convalescents	prior to release
light	4.31	4.6	-	-	5.03	5.37
semi-acute	7.22	6.87	-	-	6.33	6.2
acute	10.6	9.44	-	-	7.19	6.18
prolonged	3.27	2.6	-	-	5.79	5.41
relapsing	6.66	7.77	11.25	11.72	5.84	4.85
complicated	7.35	6.62	-	15.56	8.86	5.94